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Claims

1. A method for sub-typing, classifying, identifying or monitoring an Equine herpesvirus type 1 (EHV-1) isolate, the method comprising use of a genetic marker shown in Table 5.
2. A method as claimed in claim 1 for sub-typing the EHV-1 isolate into one of the 6 groups shown in Figure 1.
3. A method as claimed in claim 2 wherein the marker is an ORF68 polymorphic sequence shown in Table 5.
4. A method for assessing the virulence of an Equine herpesvirus type 1 (EHV-1) or type 4 (EHV-4) isolate, the method comprising use of a genetic marker.
5. A method as claimed in claim 4 wherein the marker is a DNA polymerase (ORF30) marker.
6. A method for assessing the virulence of a herpesvirus isolate, the method comprising use of virulence marker corresponding to an DNA polymerase ORF30-m1 region virulence marker.
7. A method as claimed in claim 6 wherein the herpesvirus isolate is an Equine herpesvirus type 1 (EHV-1) or type 4 (EHV-4) isolate.
8. A method as claimed in claim 6 wherein the marker is a polymorphic marker.
9. A method as claimed in claim 6 which is used to assess neurovirulence.
10. A method as claimed in claim 6 wherein a marker corresponding to ORF30 amino acid position 752 and/or 760 of the EHV-1 V592 is assessed.
11. A method as claimed in claim 10 wherein the presence of an

acidic amino acid at the position corresponding to 752 and/or a non-acidic amino acid at the position corresponding to 760 is correlated with higher virulence of the herpesvirus.

12. A method as claimed in claim 11 wherein the acidic amino acid is Asp and non-acidic amino acid is Gly.

13. A method as claimed in claim 12 wherein the nucleotide sequence of the codon encoding the amino acid is to be assessed

14. A method as claimed in claim 13 wherein the nucleotide position corresponding to ORF30 2254 and/or 2279 of the EHV-1 V592 is assessed.

15. A method as claimed in claim 14 wherein the presence of a 'G' at position 2254 and/or 2279 is correlated with higher virulence of the herpesvirus.

16. A method as claimed in claim 15 wherein the presence of a 'G' at position 2254 is correlated with higher virulence.

17. A method as claimed in claim 6 which comprises the steps of:  
(i) providing a sample of nucleic acid from the herpesvirus isolate, and,  
(ii) establishing the presence or identity of the marker in the nucleic acid sample.

18. A method as claimed in claim 17 wherein the isolate is harboured as a latent virus within host cells, wherein said host cells are lysed to release nucleic acid molecules.

19. A method as claimed in claim 17 which comprises the step of amplifying a portion of the sample of nucleic acid containing the marker prior to determining the presence or absence of the marker variant.

20. A method as claimed in claim 19, which method comprises the steps of:

(i) providing a sample of nucleic acid from the herpesvirus

isolate, and,

- (b) contacting the nucleic acid molecules with oligonucleotide primers under suitable conditions permitting hybridization of the oligonucleotides to the nucleic acid molecules; wherein a first oligonucleotide selectively binds the nucleic acid on the 3' side of the marker, and a second oligonucleotide binds to nucleic acid on the 5' side of the marker,
- (c) enzymatically amplifying a specific region of the nucleic acid molecules comprising the marker sequence using said oligonucleotides as primers;
- (d) determining whether the identity of the marker variant in the amplified sequences.

21. A method as claimed in claim 20 wherein at least one primer is a mutant oligonucleotide primer which introduces a mutation into the amplification product, such that a restriction site is created when one of the marker variants is present but not when another marker variant is present.

22. A method as claimed in claim 17 which comprises determining the binding of an oligonucleotide probe to the nucleic acid sample or amplification product therefrom, which probe comprises a nucleic acid sequence which binds specifically to one marker variant and does not bind specifically to other marker variant.

23. A method as claimed in claim 17 wherein the presence or identity of the marker variant in the nucleic acid sample is established by single strand conformation polymorphism analysis (SSCP); heteroduplex analysis; or RFLP analysis.

24. A method as claimed in claim 17 wherein the marker variant is confirmed by nucleotide sequencing.

25. A pair of oligonucleotide primers for use in probing or amplification of nucleotide positions 2254 and/or 2279 of the EHV-1 or EHV-4 ORF30-m1 region, said primers being between about 18 and 30 nucleotides in length, which pair consists of a first oligonucleotide which selectively binds to nucleic acid on the 3' side of the region and a second oligonucleotide which selectively

binds to the 5' side of the region such as to be capable of amplifying a region of between 30 to 600 nucleotides

26. A pair of oligonucleotide primers as claimed in claim 25 which amplify at least the entire ORF30-m1 region.

27. A pair of oligonucleotide primers for use in probing or amplification of a region comprising any two or more of nucleotide positions 336, 344, 629, 710, 713, 719, 731-740, 755 of EHV-1 ORF68, said primers being between about 18 and 30 nucleotides in length, which pair consists of a first oligonucleotide which selectively binds to nucleic acid on the 3' side of the region and a second oligonucleotide which selectively binds to the 5' side of the region such as to be capable of amplifying a region of between 30 to 600 nucleotides

28. An oligonucleotide primer selected from the group consisting of the primers shown in SEQ. ANNEX 2.

29. A primer as claimed in claim 28 which is listed as ORF30f, ORF30r or ORF30s.

30. A primer as claimed in claim 28 which is listed as ORF68f, ORF68r, ORF68s1, ORF68s2 or ORF68s3.

31. A kit for assessing the virulence of herpesviruses, the kit containing a pair of oligonucleotide primers as claimed in claim 25, said kit further including one or more of the following: the reaction buffer for the respective method of enzymatic amplification, plus one or more oligonucleotides specific for an EHV marker labeled with a detectable moiety.

32. A kit for assessing the virulence of herpesviruses, the kit containing a pair of oligonucleotide primers as claimed in claim 26, said kit further including one or more of the following: the reaction buffer for the respective method of enzymatic amplification, plus one or more oligonucleotides specific for an EHV marker labeled with a detectable moiety.

33. A kit for typing EHV-1 isolates, the kit containing a pair of oligonucleotide primers as claimed in claim 27, said kit further including one or more of the following: the reaction buffer for the respective method of enzymatic amplification, plus one or more oligonucleotides specific for an EHV marker labeled with a detectable moiety.

34. A method for preparing a recombinant herpesvirus vaccine strain, which method includes the steps of:

- (i) providing nucleic acid from a herpesvirus genome,
- (ii) modifying the DNA POL (ORF30) gene of the herpesvirus to reduce the virulence of the gene product of said gene, wherein said modification is selected from the group consisting of insertions, substitutions, and deletion of one or more virulence markers within the region of the gene corresponding to the ORF30-m1 region of Equine herpesvirus type 1 (EHV-1) V592, and
- (iii) combining the modified virus encoded by the genome with a pharmaceutically acceptable diluent, adjuvant, or carrier,

35. A method as claimed in claim 34 wherein modification provides a non-acidic amino acid at the position corresponding to position 752 and/or an acidic amino acid at the position corresponding to position 760 of the region.

36. A recombinant herpesvirus strain obtainable by the method of claim 34.

37. A live viral vaccine comprising an immunogenically effective amount of the herpesvirus strain of claim 36 in a pharmaceutically acceptable carrier.

38. A vaccine as claimed in claim 37 in dosage unit form, said dosage unit being adapted to provide from  $10^3$  to  $10^8$  TCID<sub>50</sub> of the recombinant herpesvirus strain per host.

39. A method for immunizing a host against a herpesvirus disease which method includes a step of inoculating the host with an immunity-inducing dose of a vaccine as claimed in claim 37.

40. A method for immunizing a host against a herpesvirus disease which method includes a step of inoculating the host with an immunity-inducing dose of a vaccine as claimed in claim 38.

41. An isolated peptide comprising a contiguous portion of at least 10, 15, 20, 30, 40, or 50 amino acids of the amino acid sequence of the ORF30 sequence of an Equine herpesvirus type 1 (EHV-1) isolate, wherein the peptide includes at least a portion corresponding to positions 752-760 of SEQ. ANNEX 3.

42. Use of the sequence of the Equine herpesvirus type 1 (EHV-1) strain V592 polymerase ORF30-m1 region in the provision of a genetic marker for assessing the virulence of a herpesvirus, which method comprises:

- (i) comparing the sequence of said region with the corresponding sequence in a virulent herpesvirus strain,
- (ii) identifying marker identities which differ between said sequences,
- (iii) selecting from said differing marker identities, a marker which is present in said virulent herpesvirus strain sequence, but does not co-segregate with markers indicative of the sub-type or identity of the virulent herpesvirus strain.